INTRODUCTION

Preterm birth is the leading cause of early neonatal death worldwide (Liu et al. 2012). Spontaneous preterm birth, either due to preterm labour or preterm prelabour rupture of fetal membranes (pPROM), is the single biggest contributor (Goldenberg et al. 2008). There is no safe treatment that can definitively stop preterm labour. In part, this is because the mechanisms regulating the processes of human labour and delivery are not fully understood. A greater understanding of these mechanisms is therefore imperative in order to develop any new therapeutics.

Nevertheless, what we do know is that preterm and term labour share a ‘common terminal pathway’ of increased myometrial contractility, cervical ripening and rupture of fetal membranes (Romero et al. 2006). It is now generally accepted that preterm birth results from pathological activation of this common terminal pathway of labour (Romero et al. 2006). There are many causes of preterm birth including infection, inflammation, uteroplacental ischaemia or haemorrhage, uterine overdistension or stress (Goldenberg et al. 2008). Infection and inflammation has been casually linked to early preterm birth, accounting for up to 40% of all cases (Goldenberg et al. 2008). The role of inflammation in the processes of labour have also been well established using in vitro human studies (Pavan et al. 2003, Sato et al. 2003, Lim et al. 2013c, 2014a, 2017a) and in vivo animals models (Elovitz & Mrinalini 2004, Sadowsky et al. 2006, Ilievski et al. 2007, Burd et al. 2012, Chin et al. 2016) of preterm birth. These model systems have used pro-inflammatory cytokines and Toll-like receptor (TLR) ligands to generate the hallmarks of inflammation-induced preterm birth. These include the upregulation of pro-inflammatory cytokines (e.g. IL1A, IL1B, IL6); chemokines (e.g. CXCL1, CXCL8 and CCL2): the adhesion molecule ICAM1; the contraction-associated proteins cyclooxygenase (COX)-2 (PTGS2) and the PGF3α receptor (PTGFR); the uteronic prostaglandin PGF3α and the extracellular matrix (ECM)-degrading enzyme matrix metalloproteinase 9 (MMP9) (Brodt-Eppley & Myatt 1999, Slater et al. 1999, Roh et al. 2000, Osman et al. 2003, Vadillo-Ortega & Estrada-Gutierrez 2005, Lim et al. 2014a).

Parkinson protein 7 (PARK7, also called DJ-1) is a 189 amino-acid protein of approximately 23 kD. It is ubiquitously expressed in a wide variety of cell types...
where it predominantly exists as a homodimer in the cytoplasm. PARK7 was first identified as an oncogene (Nagakubo et al. 1997); however, it possesses multiple functions including modulation of oxidative stress (Clements et al. 2006), cell death (Junn et al. 2005) and inflammation (Kim et al. 2013). It has been implicated in many diseases, most notably Parkinson’s disease (Bonifati et al. 2003) and cancer (MacKeigan et al. 2003, Hod 2004). More recently, PARK7 has been found to be involved in diabetes (Waanders et al. 2009, Jain et al. 2012, Kim et al. 2014) and liver disease (Chen et al. 2016). In vitro, increased PARK7 expression has been reported in pancreatic islets by hyperglycaemia (Waanders et al. 2009) and in chemical murine models of liver injury (Chen et al. 2016).

With respect to inflammation, PARK7 has been reported to be both anti-inflammatory and pro-inflammatory. In particular, the anti-inflammatory actions of PARK7 have been demonstrated in brain cells associated with Parkinson’s disease pathogenesis. For example, in culture from PARK7-knockout mice, there is increased expression of inflammatory mediators in response to IFN-γ (Kim et al. 2013) or LPS (Chen et al. 2016), while LPS-induced expression of pro-inflammatory cytokines is increased in PARK7-deficient astrocytes (Waak et al. 2009) and microglia (Trudler et al. 2014). On the other hand, increasing evidence from both in vitro and in vivo models suggest that PARK7 exerts pro-inflammatory actions. In mouse models of disease, there are significantly fewer infiltrating neutrophils and macrophages, and the expression of inflammatory genes (such as IL1B, IL6, CCL2 and TNF) is lower in the liver, lung, adipose tissue and brain of PARK7-knockout mice compared to wild-type mice (Waak et al. 2009, Nguyen et al. 2013, Kim et al. 2014, Liu et al. 2015, Chen et al. 2016, Yu et al. 2016, Lim et al. 2017a, Qiu et al. 2017). Similarly, liver cells from PARK7-knockout mice also have a decreased chemokine expression, which limits their ability to recruit macrophages (Chen et al. 2016). PARK7 deficiency in 3T3-L1 adipocyte cells results in reduced expression of pro-inflammatory cytokines (Kim et al. 2014), and there is a blunted response to TLR signalling in PARK7-deficient macrophages (Liu et al. 2015).

Increased PARK7 expression has been reported in placentas of women with hepatitis B virus (Chung et al. 2013) and gestational trophoblastic disease (Zhang et al. 2010). There are, however, no studies on PARK7 in relation to human labour. Thus, in this study, we sought to characterise the expression of PARK7 in myometrium and fetal membranes from non-labouring and labouring women. Furthermore, the effect of PARK7 siRNA knockdown on cytokine- and TLR-induced expression and secretion of pro-inflammatory and pro-labour mediators in human primary myometrial and amnion cells was also assessed. In order to induce pro-inflammatory and pro-labour mediators associated with preterm birth (Gillaux et al. 2011, Hoang et al. 2014, Lim et al. 2014a), the following were used: pro-inflammatory cytokines IL1B and TNF; the TLR ligands and bacterial products fibroblast-stimulating lipopeptide (isl-1; TLR2/6 ligand) and flagellin (TLR5 ligand); and TLR3 ligand and dsRNA analogue polyinosinic:polycytidylic acid (poly(I:C)).

**Materials and methods**

**Ethics statement**

The Research Ethics Committee of Mercy Hospital for Women approved this study (R04-29). Written, informed consent was obtained from all participating women.

**Tissue collection and preparation**

Myometrium was obtained from the upper margin of the lower uterine segment incision during Caesarean section. Placenta (with attached fetal membranes) was obtained from pregnant women at the time of Caesarean section or after vaginal delivery. All tissue samples were brought to the research laboratory and processed within 15 min of delivery. Fetal membranes and myometrial tissues were washed in PBS to remove excess blood. Tissues were then cleared of serosa, fibrous or damaged tissue and visible blood vessels. Fetal membranes and myometrial tissues were dissected into smaller pieces, and immediately snap frozen in liquid nitrogen and stored at −80°C for the expression studies or used immediately for cell culture experiments.

For all studies, women with any underlying medical conditions such as diabetes, asthma, polycystic ovary syndrome, preeclampsia and macrovascular complications were excluded. Additionally, women with multiple pregnancies, obese women and foetuses with chromosomal abnormalities were excluded.

**Tissue collection for expression studies**

For the expression studies, myometrium and fetal membranes were obtained from women at the time of delivery at term or preterm as detailed below. All tissues were brought to the research laboratory, processed within 15 min of delivery, and samples were immediately snap frozen in liquid nitrogen and stored at −80°C.

Myometrial biopsies, obtained from the upper margin of the lower uterine segment incision during Caesarean section, were collected from (i) women undergoing elective Caesarean section in the absence of labour (n = 8 patients; mean gestational age 39.4 ± 0.3 weeks) and (ii) women who delivered during active labour; labour was defined as the presence of regular uterine contractions (every 3–4 min) resulting in cervical effacement and dilation (n = 8 patients; mean gestational age 39.8 ± 0.2 weeks). Indications for Caesarean section in the absence of labor were breech presentation and/or previous Caesarean section. Indications for Caesarean section in the labouring samples were for placenta praevia, fetal distress and delayed or failure to progress. In the labouring group, none of
the patients received any medications to augment or induce labour, and the average length of labour was 10 h ± 6 h 40 min.

Fetal membranes were obtained from women at (i) term, no labour undergoing elective Caesarean section (indications for Caesarean section were breech presentation and/or previous Caesarean section) (n = 9 patients; mean gestational age 39.3 ± 0.3 weeks) and (ii) term after spontaneous labour, spontaneous membrane rupture and normal vaginal delivery (n = 9 patients; mean gestational age 40.4 ± 0.2 weeks). Fetal membranes from the non-labouring group were obtained from the supracervical site (SCS). Identification of the SCS was performed as previously detailed (Lappas et al. 2011). In the after labour group, fetal membranes were obtained from the site of membrane rupture as previously described (Lappas et al. 2011). None of the patients received any medications to augment or induce labour, and the average ± S.E.M. length of labour was 8 h 31 min ± 2 h 7 min. The relevant clinical characteristics of the patients used for this study have previously been described (Lim et al. 2017a).

Fetal membranes were also obtained from women at preterm birth for two studies: preterm labour and preterm chorioamnionitis. For these two studies, different patients were used, with the clinical details of the patients detailed elsewhere (Lim et al. 2017a). For the preterm labour study, fetal membranes (amnion and choriondecidua) were obtained from women at (i) Caesarean section in the absence of labour with intact membranes (n = 9 patients; mean gestational age 33.3 ± 0.8 weeks) and (ii) after spontaneous labour and normal vaginal delivery (n = 9 patients; mean gestational age 33.6 ± 0.7 weeks). For the chorioamnionitis study, only amnion was collected as the choriodicidal tissue was degraded and could not be collected. Amnion was collected from (i) Caesarean section in the absence of labour (n = 8 patients; mean gestational age 33.6 ± 0.7 weeks) and (ii) Caesarean section in the absence of labour with histologically confirmed acute chorioamnionitis (n = 8 patients; mean gestational age 28.5 ± 1.5 weeks). Indications for preterm delivery (in the absence of labour) were placenta prævia, placental abruption, antepartum haemorrhage (APH) or Rhesus isoimmunisation. All placentas collected from preterm gestations were subject to histopathological examination, and fetal membranes were swabbed for microbiological culture investigations. Chorioamnionitis was diagnosed pathologically according to standard criteria, which included histological evidence of macrophages and neutrophils permeating the chorionic cell layer and often infiltrating the amniotic cell layer (Tita & Andrews 2010).

**Primary amnion and myometrial cell culture**

For these studies, fresh amnion and myometrium were obtained from women who delivered healthy, singleton infants at term (37–40 weeks gestation) undergoing elective Caesarean section in the absence of labour. Amnion epithelial cells were prepared as previously described (Lim et al. 2013b). Briefly, amnion strips were washed in PBS and digested, twice, with 0.125% collagenase A and 0.25% trypsin in serum-free DMEM for 35 min at 37°C. The cell suspension was filtered through a cell strainer and the eluate was neutralised with 1% fetal calf serum (FCS). The cell suspensions were centrifuged at 500 g for 10 min and the cells cultured in DMEM/F-12, 10% heat-inactivated FCS and 100 U/mL penicillin G and 100 mg/mL streptomycin. The media was replaced after 4 h then every 24–48 h thereafter. Amnion cells from passage 1 were used. Myometrial cells were isolated and cultured as previously described (Lim et al. 2013a). Briefly, myometrium was minced and digested for 1 h in Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (DMEM/F-12) with 3 mg/mL type 1 collagenase (Worthington Biochemical, Freehold, NJ, USA) and 80 μg/mL DNase 1 (Roche Diagnostics) at 37°C. Cells were centrifuged at 400 g for 10 min and grown in DMEM/F-12 enriched with 10% heat-inactivated FCS (containing 100 U/mL penicillin G and 100 mg/mL streptomycin). Myometrial cells from passage 1 to 8 were used.

Transfection of primary amnion and myometrial cells with siRNA was performed as previously described (Lim et al. 2015). Briefly, cells at approximately 50% confluence were transfected using Lipofectamine 3000 according to manufacturer’s guidelines (Life Technologies). PARK7 siRNA (siPARK7) and negative control siRNA (siCONT) were obtained from Ambion (Thermo Fisher Scientific). Cells were transfected with 50 nM siPARK7 or 50 nM siCONT in DMEM/F-12 for 48 h. The medium was then replaced with DMEM/F-12 (containing 0.5% BSA) with or without 1 ng/mL IL1B (PeproTech; Rocky Hill, NJ, USA), 10 ng/mL TNF (PeproTech), 250 ng/mL fsl-1 (InVivoGen; San Diego, California, USA), 1 μg/mL flagellin (purified flagellin from Salmonella typhimurium; InVivoGen) or 5 μg/mL poly(I:C) (Sigma) and the cells were incubated at 37°C for an additional 20 h. After final incubation, cells and media were collected separately and stored at −80°C. Cell viability was assessed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) proliferation assay as previously described (Lim et al. 2014b). The response to IL1B, TNF, fsl-1, flagellin and poly(I:C) between patients varied greatly, as previously reported (Lim et al. 2015). Thus, data are presented as fold change in expression relative to the expression level in IL1B-, TNF-, fsl-1-, flagellin- or poly(I:C)-stimulated siCONT-transfected cells. Experiments were performed in amnion and myometrial cells obtained from six patients.

**NF-κB RELA luciferase assay**

A luciferase assay was also used to determine the effect of siPARK7 on NF-κB RELA transcriptional activity as previously described (Lim et al. 2016b). Briefly, primary myometrial cells were transfected with 300 ng/mL RELA reporter construct (Qiagen) using FuGENE HD transfection reagent (Promega). After 6 h, cells were transfected with 50 nM of siPARK7 or siCONT (as detailed above) for 48 h. The medium was then replaced with DMEM/F-12 (containing 0.5% BSA), with or without 1 ng/mL IL1B, 10 ng/mL TNF, 250 ng/mL fsl-1, 1 μg/mL flagellin or 5 μg/mL poly(I:C), and the cells were incubated at 37°C for an additional 20 h. After final incubation, cells were harvested in lysis buffer, and luminescence activity was measured using a luciferase reporter assay kit (Life Research; Scoresby, Vic, Australia) and Renilla luciferase flash assay kit (Thermo Fisher Scientific) as instructed. The ratio of the firefly


Downloaded from Bioscientifica.com at 04/03/2019 05:42:10PM via free access
luciferase level to the Renilla luciferase level was determined and the results are expressed as a ratio of normalised luciferase activity. The experiments were performed from myometrium obtained from six patients.

RNA extraction and qRT-PCR
RNA extractions and qRT-PCR was performed as previously described (Lappas 2015). RNA concentration and purity were measured using a NanoDrop ND1000 (Thermo Fisher Scientific). RNA was converted to cDNA using the high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. The RT-PCR was performed using the CFX384 Real-Time PCR detection system (Bio-Rad Laboratories) using 100 nM of pre-designed and validated QuantiTect primers (primer sequences not available) (Qiagen). Average gene Ct values were normalised to the average YWHAZ and succinate dehydrogenase (SDHA) Ct values of the same cDNA sample. Fold differences were determined using the comparative Ct method.

Enzyme immunoassays
Assessment of cytokine and chemokine release of IL6 and CXCL8 was performed using the CytoSet sandwich ELISA according to the manufacturer’s instructions (LifeTechnologies). The release of CCL2 and sICAM1 was performed by sandwich ELISA from R&D Systems according to the manufacturer’s instructions. The release of PGF$_2\alpha$ into the incubation medium was assayed using a commercially available competitive enzyme immunoassay kit according to the manufacturer’s specifications (Cayman Chemical Company). The interassay and intraassay coefficients of variation for all assays were less than 10%.

Statistical analysis
All statistical analyses were undertaken using GraphPad Prism (GraphPad Software). For two sample comparisons, either a paired or unpaired Student’s t-test was used to assess statistical significance between normally distributed data; otherwise, the nonparametric Mann–Whitney U (unpaired) or the Wilcoxon (matched pairs) tests were used. For all other comparisons, the homogeneity of data was assessed by the Bartlett’s test, and when significant, the data were logarithmically transformed before further analysis using a one-way ANOVA (with LSD post hoc testing to discriminate among the means). Statistical significance was ascribed to a $P$ value $\leq 0.05$. Data were expressed as mean ± S.E.M.

Results
Expression of PARK7 in human myometrium and fetal membranes from non-labouring and labouring women
The first aim was to characterise the expression of PARK7 in myometrium and fetal membranes obtained from term and preterm deliveries that have undergone labour. At term, significantly higher PARK7 mRNA expression was observed in myometrium (Fig. 1A) and fetal membranes after spontaneous labour (Fig. 1B) when compared to non-labouring tissues. At preterm, however, there was no difference in PARK7 mRNA expression between fetal membranes obtained from non-labouring women and fetal membranes obtained from women after spontaneous labour (Fig. 1C). On the other hand, when compared to the preterm group without histologic chorioamnionitis, the mRNA expression of PARK7 was significantly higher in the preterm group with histologic chorioamnionitis. Several attempts to quantify PARK7 protein with commercially available antibodies were unsuccessful.

Effect of siPARK7 on pro-inflammatory cytokines and chemokines in primary myometrial and amnion cells
Supplementary Figure 1 (see section on supplementary data given at the end of this article) depicts the efficacy of siPARK7 transfection. In both myometrial and amnio
cells, siPARK7 decreased PARK7 mRNA expression by ~95%. There was no effect of siPARK7 on cell viability as determined by MTT cell viability assay.

For all the following experiments, myometrial cells were treated with IL1B, TNF, fsl-1, flagellin or poly(I:C), while amnion cells were treated with IL1B only. These treatments were chosen as they represent models of inflammation associated with term and preterm labour. The effect of siPARK7 on the expression of pro-inflammatory cytokines and chemokines in myometrial cells is shown in Figs 2 and 3. Myometrial cells transfected with siCONT and treated with IL1B or TNF significantly increased pro-inflammatory cytokine IL6, and chemokines CXCL2 and CCL2 mRNA expression and release (Fig. 2). Myometrial cells transfected with siPARK7 significantly decreased IL1B-induced IL6 and CCL2 mRNA expression and release. There was no change in IL1B-induced CXCL8 mRNA expression; however, there was a significant decrease in the release of CXCL8 in siPARK7 cells. In siPARK7 cells, there was a significant decrease in TNF-induced CXCL8 and CCL2 mRNA expression and release. There was also a decrease in TNF-induced IL6 secretion in siPARK7 cells; however, the decrease in TNF-induced IL6 mRNA expression was not statistically significant. Myometrial cells transfected with siCONT and treated with fsl-1, flagellin and poly(I:C), as expected, significantly increased IL6, CXCL8 and CCL2 mRNA expression and release (Fig. 3). Cells transfected with siPARK7 significantly decreased both fsl-1 and poly(I-C)-induced IL6, CXCL8 and CCL2 mRNA expression and secretion. Similarly, there was a decrease in flagellin-induced IL6 and CXCL8 mRNA expression and release. siPARK7 cells also decreased flagellin-induced CCL2 mRNA expression, but not CCL2 secretion.

In amnion cells, the effect of siPARK7 on the expression of pro-inflammatory cytokines and chemokines is shown in Fig. 4. Treatment with IL1B significantly increased IL6, CXCL8 and CCL2 mRNA expression and secretion. siPARK7-transfected cells showed a decrease in IL1B-induced IL6 and CXCL8 mRNA expression and secretion, but there was no effect on CCL2 mRNA expression and secretion.

**Effect of siPARK7 on the adhesion molecule ICAM1 in primary myometrial and amnion cells**

The effect of siPARK7 on the expression of ICAM1 in amnion and myometrial cells is shown in Fig. 5. Treatment of IL1B significantly increased ICAM1 mRNA expression and ICAM1 secretion in amnion cells (Fig. 5C and D). There was a significant decrease in IL1B-induced ICAM1 mRNA expression and secretion in siPARK7 amnion cells. In myometrial cells, treatment with IL1B, TNF, fsl-1, flagellin and poly(I:C) all significantly increased ICAM1 mRNA expression and secretion (Fig. 5A, B, E, F, G, H, I, J, K and L). The effect of siPARK7 in myometrial cells was a significant decrease in ICAM1 mRNA expression and secretion by all treatments.
Figure 3: Effect of siPARK7 on pro-inflammatory cytokines and chemokines induced by TLR ligands in primary myometrial cells. Human primary myometrial cells were transfected with 50 nM siCONT or 50 nM siPARK7 for 48 h and then treated with (A, B, G, M and N) 250 ng/mL fsl-1, (C, D, I, J, O and P) 1 μg/mL flagellin (flag) or (E, F, K, L, Q and R) 5 µg/mL poly(I:C) for an additional 20 h (n = 5 patients). (A, C, E, G, I, K, M, O and Q) IL6, CXCL8 and CCL2 mRNA expression was analysed by qRT-PCR. (B, D, F, H, J, L, N, P and R) The concentration of IL6, CXCL8 and CCL2 in the incubation medium was assayed by ELISA. For all data, the fold change was calculated relative to fsl-1-, flag- or poly(I:C)-stimulated siCONT-transfected cells and displayed as mean ± s.e.m. ***P ≤ 0.001 vs fsl-1-stimulated siCONT-transfected cells; §P ≤ 0.05 vs flag-stimulated siCONT-transfected cells; #P ≤ 0.05 vs poly(I:C)-stimulated siCONT-transfected cells. All data were analysed by one-way repeated-measures ANOVA.

Figure 4: Effect of siPARK7 on pro-inflammatory cytokines and chemokines induced by IL1B in primary amnion cells. Human primary amnion cells were transfected with 50 nM siCONT or 50 nM siPARK7 for 48 h and then treated with 1 ng/mL IL1B for an additional 20 h (n = 5 patients). (A, C and E) IL6, CXCL8 and CCL2 mRNA expression was analysed by qRT-PCR. (B, D and F) The concentration of IL6, CXCL8 and CCL2 in the incubation medium was assayed by ELISA. For all data, the fold change was calculated relative to IL1B-stimulated siCONT-transfected cells, and displayed as mean ± s.e.m. *P ≤ 0.05 vs IL1B-stimulated siCONT-transfected cells (one-way repeated-measures ANOVA).
Effect of siPARK7 on the PTGS2-prostaglandin pathway in primary myometrial cells

Primary myometrial cells treated with IL1B and TNF were used to determine the effect of siPARK7 on the expression of mediators in the PTGS2-prostaglandin pathway. Treatment with flsl-1, flagellin and poly(I:C) did not elicit a high enough response to perform analysis. Cells transfected with siCONT and treated with IL1B or TNF, significantly increased PTGS2 and PTGFR mRNA expression and secretion of PGF2α (Fig. 6). There was no effect of siPARK7 on IL1B or TNF-induced PTGS2 mRNA expression; however, there was a significant decrease in IL1B and TNF-induced PTGFR mRNA expression and secretion of PGF2α in siPARK7-transfected cells.

Figure 5 Effect of siPARK7 on ICAM1 in primary myometrial and amnion cells. Human primary (A, B, E, G, H, I, J, K and L) myometrial and (C and D) amnion cells were transfected with 50 nM siCONT or 50 nM siPARK7 for 48 h and then treated with (A, B, C and D) 1 ng/mL IL1B, (E and F) 10 ng/mL TNF, (G and H) 250 ng/mL flsl-1, (I and J) 1 µg/mL flagellin (flag) or (K and L) 5 µg/mL poly(I:C) for an additional 20 h (n = 5 patients). The concentration of ICAM1 in the incubation medium was assayed by ELISA. For all data, the fold change was calculated relative to IL1B-, TNF-, flsl-1- or flag-stimulated siCONT-transfected cells, and displayed as mean ± S.E.M. *P ≤ 0.05 vs IL1B-stimulated siCONT-transfected cells; **P ≤ 0.05 vs TNF-stimulated siCONT-transfected cells; ***P ≤ 0.05 vs flsl-1-stimulated siCONT-transfected cells; §P ≤ 0.05 vs flag-stimulated siCONT-transfected cells; #P ≤ 0.05 vs poly(I:C)-stimulated siCONT-transfected cells. All data were analysed by one-way repeated-measures ANOVA.

Figure 6 Effect of siPARK7 on PTGS2-prostaglandin pathway induced by IL1B or TNF in primary myometrial cells. Human primary myometrial cells were transfected with 50 nM siCONT or 50 nM siPARK7 for 48 h and then treated with (A, B and C) 1 ng/mL IL1B or (D, E and F) 10 ng/mL TNF for an additional 20 h (n = 5 patients). (A, B, D and E) PTGS2 and PTGFR mRNA expression was analysed by qRT-PCR. (C and F) The concentration of PGF2α in the incubation medium was assayed by ELISA. For all data, the fold change was calculated relative to TNF-stimulated siCONT-transfected cells, and displayed as mean ± S.E.M. *P ≤ 0.05 vs IL1B-stimulated siCONT-transfected cells; **P ≤ 0.05 vs TNF-stimulated siCONT-transfected cells. All data were analysed by one-way repeated-measures ANOVA.
Effect of siPARK7 on NF-κB RELA transcriptional activity in primary myometrial cells

A luciferase assay was used to determine the effect of siPARK7 on NF-κB RELA transcriptional activity. Cells treated with IL1B, TNF, fsl-1, flagellin and poly(I:C) all significantly increased NF-κB RELA transcriptional activity (Fig. 7). There was no effect of siPARK7 on IL1B or TNF-induced NF-κB activity; however, in siPARK7 transfected cells, there was a significant decrease in fsl-1, flagellin and poly(I:C)-induced NF-κB transcriptional activity.

Discussion

For the first time, we report that PARK7 is expressed in human fetal membranes and myometrium. At term, PARK7 mRNA expression was significantly higher in human fetal membranes and myometrium obtained from labouring women compared to non-labouring women. In fetal membranes at preterm, there was no change in PARK7 mRNA expression with preterm labour, but there was an increase in PARK7 mRNA expression in amnion with histologic chorioamnionitis. Accordingly, we also found that when we decreased PARK7 expression in primary human myometrial and amnion cells using siRNA, there was diminished expression and/or secretion of the pro-inflammatory cytokine IL6, the chemokines CXCL8 and CCL2 and the adhesion molecule ICAM1 in response to sterile inflammatory insults (IL1B, TNF, fsl-1, flagellin, poly(I:C)). Furthermore, in primary myometrial cells, although there was no effect of siPARK7 on PTGS2 mRNA expression, PGF₂α secretion and PTGFR mRNA expression were significantly blunted in response to IL1B or TNF treatment.

There is increasing evidence demonstrating that human labour is associated with an inflammatory response. Initial studies demonstrated leukocyte invasion into labouring myometrium and fetal membranes when compared to non-labouring tissues (Osman et al. 2003). Increased inflammation is also commonly reported in gene array studies of labouring myometrium and fetal membranes (Bollapragada et al. 2009, Weiner et al. 2010). More recently, the transcriptome of human labouring term myometrium was compared to two mouse models of preterm birth: LPS model that initiates labour via activation of uterine inflammation and progesterone withdrawal model in which labour is initiated without inflammatory gene activation (Migale et al. 2016). The transcriptome changes observed in human myometrium at the time of labour were most similar to the LPS (i.e. inflammation) mouse model of preterm birth (Migale et al. 2016), further demonstrating a central role for inflammatory pathways in human labour. In this study, PARK7 mRNA expression was increased in myometrium and fetal membranes obtained from labouring women compared to non-labouring women. Whether this is a cause or consequence of labour is not known and temporal associated changes in PARK7 expression would address this. Furthermore, in preterm fetal membranes, there was no change in PARK7 mRNA expression with labour, but there was an increase with histologic chorioamnionitis (amnion only, due to degradation of choridecidua). This increase in PARK7 mRNA expression with chorioamnionitis would suggest a correlation between PARK7 and inflammation;
no change of expression with preterm labour may be due to overall increased inflammatory processes due to the preterm gestation, with labour having no further effect, as we have previously postulated (Lim et al. 2016a,b). Nevertheless, our findings do suggest that PARK7 may play a role in the inflammatory processes associated with human labour in myometrium and fetal membranes. In order to answer this question, we performed functional studies in primary cells isolated from human myometrium or amnion and induced an inflammatory state using pro-inflammatory cytokines and TLR ligands.

During labour, IL1B and TNF levels are increased due to the influx of leukocytes into intrauterine tissues such as myometrium and fetal membranes (Thomson et al. 1999, Osman et al. 2003). IL1B and TNF can then bind to their receptors to initiate a cascade of events that results in labour and delivery. These events include increasing the production of (i) pro-inflammatory cytokines through a positive feed-forward loop leading to an amplification of events; (ii) chemokines and adhesion molecules that are involved in leukocyte trafficking into myometrium into intrauterine tissues and (iii) contraction-associated proteins including prostaglandin-endoperoxide synthase 2 (PTGS2) and prostaglandin receptors such as PTGFR and the urotectone prostaglandins such as PGF$_{2\alpha}$ (Bartlett et al. 1999, Erkinheimo et al. 2000, Rauk & Chiao 2000). IL1B has also been shown to increase the contractile potential of myometrial smooth muscle (Tribe et al. 2003). In vivo, these pro-inflammatory cytokines can induce preterm birth and uterine activation (Yoshimura & Hirsch 2005, Sadowsky et al. 2006, Nadeau-Vallée et al. 2015). In order to elucidate a role for PARK7 in the genesis of pro-labour mediators induced by TNF and IL1B, primary myometrial and amnion cells were transfected with siPARK7. In primary myometrial cells treated with TNF or IL1B, the expression and/or secretion of the pro-inflammatory cytokines IL6, the chemokines CXCL8 and CCL2, the adhesion molecule ICAM1, PGF$_{2\alpha}$ and its receptor PTGFR was lower in cells transfected with siPARK7. Surprisingly, although IL1B- and TNF-induced secretion of PGF$_{2\alpha}$ and mRNA expression of its receptor PTGFR were significantly suppressed in siPARK7-transfected cells, there was no change in PTGS2 mRNA expression. PTGS2 converts arachidonic acid to PGH2, which is the common precursor for the generation of all primary prostaglandins by cell-specific isomerases and synthases; PGF$_{2\alpha}$ is generated by prostaglandin F synthase (FAM213B). Thus, it is possible that PARK7 regulates PGF$_{2\alpha}$ by acting on FAM213B. In primary amnion cells, in the presence of IL1B, siPARK7-transfected cells showed decreased mRNA expression and secretion of IL6, CXCL8 and ICAM1 when compared to siCONT-transfected cells. Altogether, these findings indicate that PARK7 is involved in the IL1B and TNF signalling pathways associated with labour.

Infection is also frequently associated with human preterm birth, especially those deliveries before 32-week gestation (Romero et al. 2006). Interestingly, the expression of PARK7 is increased in placentas of women with hepatitis B virus (Chung et al. 2013). To complement these studies, the expression of PARK7 was found to be increased in amnion from women with preterm histologic chorioamnionitis. Inflammation is commonly caused by infection; the maternal and/or fetal response to chorioamnionitis can lead to preterm labour through the activation of the inflammatory response. Bacteria, viruses and their products bind to TLRs to induce an inflammatory cascade of events that culminates in preterm birth that is similar to that described above for IL1B and TNF. In vitro, bacteria-derived molecules such as lsl-1 and flagellin and viral dsRNA analogues such as poly(I:C) are often used to mimic this inflammation (Gillaux et al. 2011, Hoang et al. 2014, Lim et al. 2014a). Thus, it was of interest to determine if PARK7 is also involved in the genesis of pro-labour mediators induced by these TLR ligands. For the first time, we report that in human myometrial cells, PARK7 siRNA knockdown is associated with a dampening of the inflammatory response associated with the TLR2/6 ligands lsl-1, the TLR5 ligand flagellin and the TLR3 ligand poly(I:C). That is, when compared to siCONT-transfected cells, the expression and/or secretion of the pro-inflammatory cytokines IL6, the chemokines CXCL8 and CCL2, the adhesion molecule ICAM1, PGF$_{2\alpha}$ and its receptor PTGFR was lower in cells transfected with siPARK7. Collectively, these findings suggest that PARK7 is involved in TLR signalling in human myometrial cells.

PARK7 has been shown exert its effects by modulating multiple intracellular signalling molecules and transcription factors including mitogen-activated protein kinase (MAPK), protein kinase B (PKB/Akt) and NF-kB RELA (Sean McNally et al. 2011, Kim et al. 2014, Liu et al. 2015). Given the central role of NF-kB in regulating pro-inflammatory and pro-labour mediators induced by IL1B, TNF and TLR ligands (Lim et al. 2014a, 2017b,c), we examined the effect of siPARK7 on NF-kB RELA transactivation using a luciferase reporter assay. We found that, when compared with siCONT-transfected cells, siPARK7-transfected cells had markedly decreased luciferase activity after TLR activation. There was, however, no effect of siPARK7 on IL1B- or TNF-induced NF-kB RELA activation. These findings suggest that PARK7 regulates TLR-induced pro-inflammatory and pro-labour mediators via NF-kB. On the other hand, PARK7 regulates IL1B- or TNF-induced pro-inflammatory and pro-labour mediators via an NF-kB-independent mechanism. In addition to NF-kB, PARK7 has been shown to exert its effects by modulating multiple intracellular signalling molecules and transcription factors including MAPK and protein

For the first time, our studies highlight a novel role for PARK7 in human labour. Increased PARK7 expression was observed in myometrium and fetal membranes with spontaneous term labour and in preterm amnion with chorioamnionitis. Consequently, siRNA knockdown experiments in primary myometrial and amnion cells revealed that PARK7 contributes to the expression of pro-labour mediators, namely pro-inflammatory cytokines, chemokines, the adhesion molecule ICAM1, and the contraction-associated proteins PGF₂α and PTGFR. Future studies are, however, required to further elucidate the role and regulation of PARK7 in human labour and whether inhibiting PARK7 in vivo can prevent spontaneous preterm birth. Nevertheless, these findings add to our understanding of the regulation of the processes of human labour and delivery which may be beneficial in the design of efficacious therapeutic strategies to prevent preterm birth.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/REP-17-0604.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
Associate Prof. Martha Lappas is supported by a Career Development Fellowship from the National Health and Medical Research Council (NHMRC; grant no. 1047025) and Research Fellowship from the University of Melbourne. Funding for this study was provided by the NHMRC (grant no. 1058786), University of Melbourne, Norman Beischer Medical Research Foundation and the Mercy Research Foundation.

Acknowledgements
The following are gratefully acknowledged: the clinical research midwives Genevieve Christophers, Gabrielle Pell, and Rachel Murdoch for sample collection; and the Obstetrics and Midwifery staff of the Mercy Hospital for Women for their cooperation.

References


Ilievski V, Lu SJ & Hirsch E 2007 Activation of toll-like receptors 2 or 3 and preterm delivery in the mouse. Reproductive Sciences 14 315–320. (https://doi.org/10.1161/01.STR.0000257533.80607.4d)


www.reproduction-online.org


McNally RS, Davis B, Clements CM, Accavitti-Loper M, Mak TW & Ting JP 2011 DJ-1 enhances cell survival through the binding of zeazane, a negative regulator of NF-kB. Journal of Biological Chemistry 286 4098–4106. (https://doi.org/10.1074/jbc.M110.147371)


Received 5 October 2017
First decision 8 November 2017
Revised manuscript received 13 November 2017
Accepted 4 December 2017